Immunology & Cell Biology 2021; **99**: 255–273
www.wileyonlinelibrary.com/journal/icb

REVIEW

Impact of prebiotics on immune response: from the bench to the clinic

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Keywords
dendritic cells, immunomodulation, macrophage, oligosaccharide, T cells

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Received 30 March 2020; Revised 31 August 2020 and 23 September 2020; Accepted 29 September 2020

doi: 10.1111/imcb.12409

Immunology & Cell Biology 2021; **99**: 255–273

Abstract

Several preclinical and clinical studies have shown the immunomodulatory role exerted by prebiotics in regulating the immune response. In this review, we describe the mechanistic and clinical studies that decipher the cell signaling pathways implicated in the process. Prebiotic fibers are conventionally known to serve as substrate for probiotic commensal bacteria that release of short-chain fatty acids in the intestinal tract along with several other metabolites. Subsequently, they then act on the local as well as the systemic immune cells and the gut-associated epithelial cells, primarily through G-protein-coupled receptor-mediated pathways. However, other pathways including histone deacetylase inhibition and inflammasome pathway have also been implicated in regulating the immunomodulatory effect. The prebiotics can also induce a microbiota-independent effect by directly acting on the gut-associated epithelial and innate immune cells through the Toll-like receptors. The cumulative effect results in the maintenance of the epithelial barrier integrity and modulation of innate immunity through secretion of pro- and anti-inflammatory cytokines, switches in macrophage polarization and function, neutrophil recruitment and migration, dendritic cell and regulatory T-cell differentiation. Extending these *in vitro* and *ex vivo* observations, some prebiotics have been well investigated, with successful human and animal trials demonstrating the association between gut microbes and immunity biomarkers leading to improvement in health endpoints across populations. This review discusses scientific insights into the association between prebiotics, innate immunity and gut microbiome from *in vitro* to human oral intervention.

INTRODUCTION

Prebiotics are nondigestible oligosaccharides that stimulate the growth of probiotic bacteria, particularly lactobacilli and bifidobacteria.[1] Although the definition of prebiotics is debatable, the following criteria are widely used to categorize a substance as prebiotic: (1) it should be unabsorbed in the gastrointestinal tract, resistant to the acidic environment of the stomach and hydrolysis by the host enzymes, (2) it can be fermented by the resident intestinal bacteria and can selectively stimulate the growth and activity of the intestinal microbiota and (3) it should confer benefits upon host health and wellness.[2]

Some common prebiotics are oligosaccharides and can be categorized into fructans such as inulin and fructooligosaccharides (FOSs), more complex galactooligosaccharides (GOSs) and starch- and glucose-derived oligosaccharides such as resistant starch and polydextrose.[3] Prebiotics occur naturally at low concentrations, and hence some industrially important prebiotics such as FOS and GOS are also synthesized commercially at large scale. Another important category of industrially important prebiotic oligosaccharide is the human milk oligosaccharides (HMOs) that occur abundantly in human milk and are widely used in infant food formula and confer potential health benefits in neonates. They possess diverse and branched structures and can also be sialylated or fucosylated such as sialyllactose or fucosyllactose. The sialylated HMOs can anchor and inhibit sialic acid-dependent pathogen

binding to epithelial cells and thereby prevent infection.[4] The bovine milk oligosaccharide and caprine milk oligosaccharides are also being explored as alternatives to HMOs.[5] Synthetic FOS and GOS, being broadly bifidogenic and immunomodulatory, are also being tested as additives in infant food formula.[6,7] The benefits of consuming prebiotics are also ubiquitous regardless of age, gender, diet and other intrinsic and extrinsic factors. Prebiotics have been evaluated clinically and found to exhibit therapeutic potential against inflammatory disorders. Prebiotics and their potential health benefits are listed in Table 1.

Studies have now indicated that prebiotics modulate the local immune system in the gut as well as the systemic immune system.[22] The gut-associated lymphoid tissue comprises organized lymphoid tissues such as the Peyer's patches, and the mesenteric lymph nodes. The mucosal epithelium and underlying lamina propria (LP) are the effector sites that hold different immune cells including activated T cells, plasma cells, mast cells, dendritic cells (DCs) and macrophages.[23] The Paneth cells, located below the intestinal crypts, secrete antimicrobial peptides (AMPs) such as beta-defensins that can induce effective defense against the invading microorganisms.[24] Epithelial cells, in response to bacterial entry, secrete factors such as interleukin (IL)-8, monocyte chemoattractant protein 1, RANTES, tumor necrosis factor-α (TNF-α) and IL-6 that exhibit chemoattraction and proinflammatory functions.[25]

The intestinal microflora plays a significant role in the overall functioning of the immune system. The prebiotics can either directly affect the number and composition of the intestinal microflora, or their metabolites, generated after fermentation, may induce additional influence on the gut-associated lymphoid tissue.[22] More importantly, prebiotics have also been shown to exert a direct effect on the host immune response independent of the microbiota.[26] They modulate the immune response by interacting with several components of the innate and acquired immune systems. The innate immune system comprises physical barriers such as mucous membranes and cells in blood and tissue such as monocytes, macrophages, dendritic cells (DC), lymphocytes, neutrophils and natural killer (NK) cells along with soluble mediators such as complement proteins and cytokines. When the innate immune system encounters a pathogen or tissue injury, inflammation is triggered. The pattern recognition receptors present on the cell surface and in the cytoplasm of these cells sense the external insult and respond by activating signaling pathways and transcription factors such as NF-κB, activator protein (AP)1, cAMP response element-binding protein, enhancer binding protein and interferon-regulatory factor that induce genes encoding cytokines, chemokines and regulators of inflammation.[27] The prebiotics can either alone or through short-chain fatty acid (SCFA) production facilitate the anti-inflammatory response by acting through the Pattern recognition receptors (PRR) or the G-protein-coupled receptor (GPCR). This review encompasses the direct and indirect effect of prebiotics on the mediators of the immune response (Figure 1).

Table 1. Prebiotics, their source[8–10] and health benefits

	Prebiotics	Source	Health benefit
1	Inulin	Chicory, asparagus, onion, garlic, artichoke	Treating symptoms of inflammatory bowel disease, immunomodulation[11,12]
2	Fructooligosaccharide	Sugar cane, asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato and rye	Bifidogenic, immunomodulatory, anti-inflammatory, effective in reducing Crohn's disease activity[13]
3	Galactooligosaccharide	Human milk and cow milk	Bifidogenic, increases calcium absorption, improves immunity[14,15]
4	β-Glucan	Cereal grains, mushrooms, algae and yeast cell wall, other marine plants	Decreases body weight, maintains body mass index[16]; acts as an immunoadjuvant in vaccines[17]; reduces the severity of upper respiratory tract infections, controls blood pressure[18]
5	Xylooligosaccharides	Bamboo shoots, fruits, vegetables, milk, honey and wheat bran	Improves blood sugar and lipid levels in diabetes patients[19,20]
6	Arabinoxylooligosaccharides	Wheat bran	Improves digestive health, management of blood sugars and lipids, modification of immune markers[20]
7	Isomaltooligosaccharides	Starch	Controls blood glucose levels by stimulating insulin as well as the incretins[21]

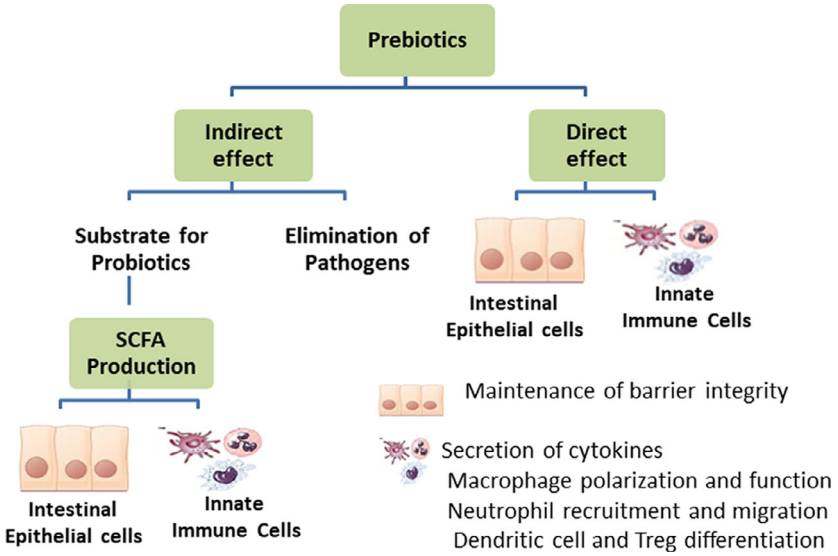

Figure 1. Effect of prebiotics on the innate immune response. The prebiotics can induce direct or indirect effect on the gut-associated epithelial and immune cells. They act as a substrate for the bacteria that leads to production of short-chain fatty acids (SCFAs) that can modulate the immune response. The prebiotic fibers can directly act on the gut epithelial and immune cells that leads to pro- or anti-inflammatory response. Treg, regulatory T cell.

PREBIOTICS MODULATE GUT BARRIER FUNCTION

Prebiotics have been reported to influence the gut barrier function by affecting the intestinal epithelial cells. The intestinal barrier and the gut-associated lymphoid tissue function as the first line of defense in innate immune responses. The intestinal epithelium is a monolayer that acts as a selectively permeable barrier against penetration of microbes, toxins or antigens. The selectivity of the barrier function is a result of the formation of complex protein–protein networks that link neighboring cells and block the intercellular space. These networks comprise three adhesive complexes, namely, desmosomes, adhesion junctions and tight junctions.[28] Any disruption in this barrier function leads to the development of inflammatory and autoimmune disease. The implication of a compromised intestinal barrier is the penetration of *Escherichia coli* lipopolysaccharide (LPS) from the gastrointestinal tract into the circulation, which leads to chronic inflammation. This inflammation causes lower absorption of nutrients in the body, mainly in children in developing countries.[29] Prebiotics have shown a favorable effect on the intestinal barrier function. An *in vitro* study by Shirai *et al.*[30] demonstrated that kestose (FOS) accelerated the recovery of epithelial tight-junction assembly in a Rho-associated kinase-dependent mechanism. The favorable effect of prebiotics has also been established on the integrity of the tight-junction proteins and other markers of gut permeability in animal models. This eventually leads to a reduction in local and systemic inflammation as indicated by reduced levels of circulatory LPS and cytokines, a shift of macrophage polarization from the M1 to M2 type and lowered hepatic expression of inflammatory and oxidative stress markers.[26,31] One of the mechanisms that improves barrier function is an increase in glucagon-like peptide production. Glucagon-like peptide, secreted by intestinal cells, which is conventionally known to increase insulin release, inhibits glucagon secretion, appetite and energy intake. Animal studies confirmed that feeding prebiotics significantly enhances glucagon-like peptide 1 or 2 and thereby controls inflammation and metabolic disorders in obesity and diabetes.[32,33] However, contradictory evidence in another study showed that feeding FOS impairs the intestinal barrier in rats and thereby increases the intestinal permeability and susceptibility to salmonella infections.[34] But, this claim was not supported by the group's own extended clinical study conducted in healthy men where no effect was observed on the intestinal permeability *per se*, although there was an increase in mucin excretion.[35] However, other dietary intervention studies with prebiotics conducted in children with type 1 diabetes[36,37] and obese adults,[38] where the intestinal barrier function is already compromised, show reduction in intestinal permeability leading to a favorable outcome.

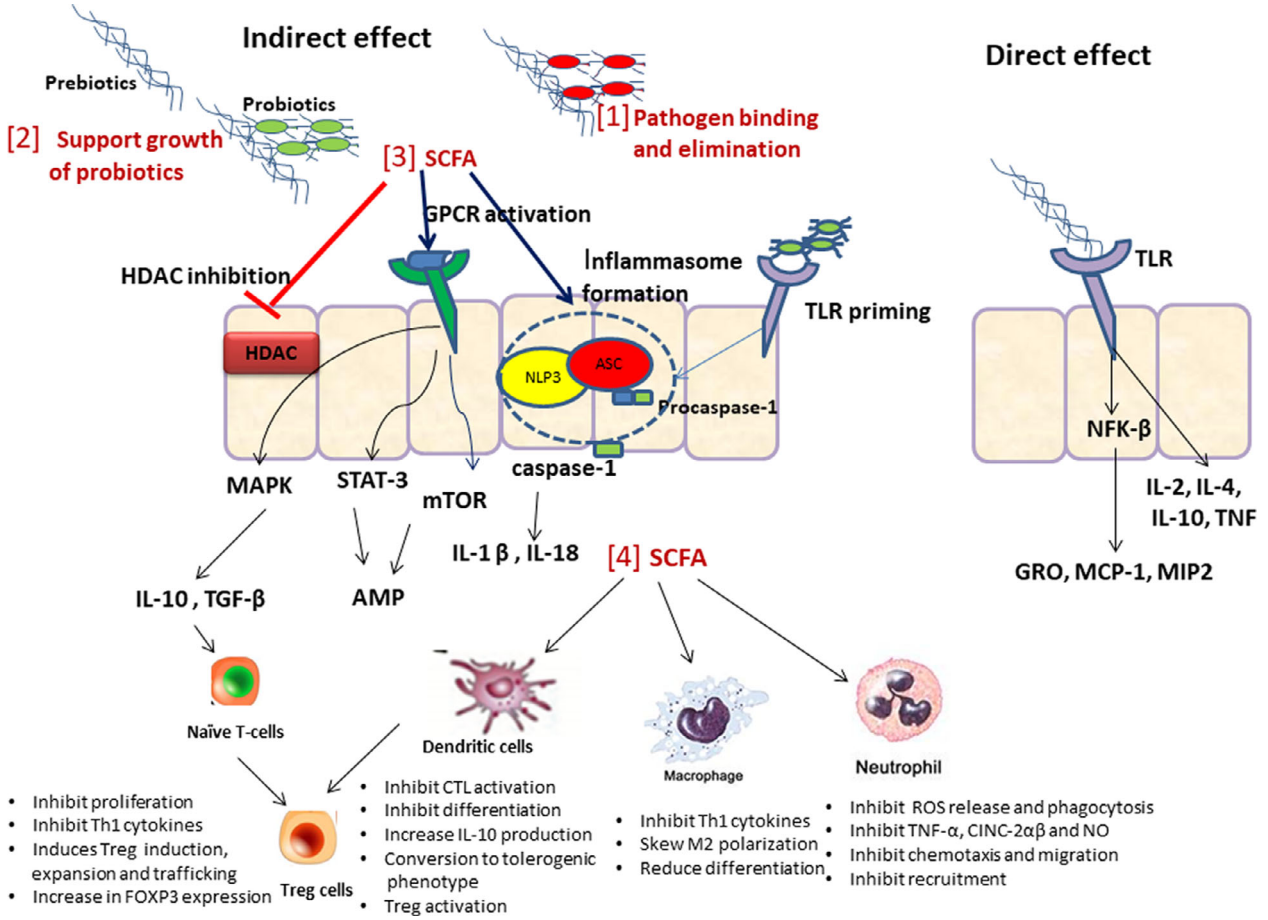

Figure 2. Direct and indirect effect of prebiotics on GALT. Indirect effect: (1) Prebiotic fibers can act as decoy receptors for pathogens and cause elimination. (2) Prebiotic fibers support the growth and activity of probiotic bacteria. (3) Prebiotic fibers are fermented by the microbes to release SCFAs. SCFAs can bind to GPCR and induce activation of MAPK, mTOR and STAT3 signaling that leads to the production of AMP and cytokines such as IL-10 and TGF-β. These cytokines skew the polarization of naïve T cell to regulatory phenotype. The SCFA can inhibit HDAC activity. SCFAs induce inflammasome complex formation by priming through TLR-4. Once primed, the NLRP3 forms the inflammasome complex in association with its adaptor protein ASC that activates caspase-1 and leads to subsequent cleavage and synthesis of functionally active IL-1β and IL-18. (4) SCFA also modulates the functionality of intestinal immune cells, namely, T cells, DCs, macrophage and neutrophils. Direct effect: The prebiotic fibers can directly act on the epithelial cells through TLR, which leads to cytokine production through NF-κB activation that eventually leads to the production of cytokines and chemokines. AMP, antimicrobial peptide; ASC, apoptosis-associated speck-like protein containing a CARD; CTL, cytotoxic T lymphocyte; DC, dendritic cell; GALT, gut-associated lymphoid tissue; GPCR, G-protein-coupled receptor; FOXP3, forkhead box P3; HDAC, histone deacetylase; IL, interleukin; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MIP, mTOR, mammalian target of rapamycin; NF-κb, nuclear factor-κB; NO, nitric oxide; NLRP3, NOD, leucin-rich repeat region and pyrin domain-containing protein; SCFAs, short-chain fatty acids; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; Th1, T helper type 1 cell; TNF, tumor necrosis factor; Treg, T regulatory cell; TLR, Toll-like receptor.

The overall findings suggest that prebiotics play a role in maintaining gut permeability and control of inflammation.

MICROBIOTA-DEPENDENT EFFECT ON GUT EPITHELIAL CELLS

There are multiple ways by which prebiotics affect gut immunity. The first step in any pathogenic invasion in the intestine is the adherence of pathogens to the epithelial cells followed by colonization. Certain prebiotics namely GOS, FOS, inulin, lactulose, raffinose, pectin oligosaccharides are reported to antagonize this effect by acting as soluble decoy receptors that mimic the pathogen-binding site, thereby facilitating the binding of the pathogen and elimination from the gut (Figure 2).[39–41]

Mechanistic studies revealed that dietary fibers, probiotics and their fermentation by-products (i.e.

SCFAs) play a role in the maintenance of epithelial barrier integrity as well as other immune functions. Cellular uptake of SCFAs primarily occurs through the GPCR signaling receptors including GPCR41 (free fatty acid receptor 3; *FFAR3*), GPCR43 (free fatty acid receptor 2; *FFAR2*) and GPCR109A (hydroxyl-carboxylic acid receptor 2; *HCAR2*).[42] Other mechanisms of SCFA uptake such as passive diffusion across the cell membrane or via soluble transporters such as the proton-coupled monocarboxylate transporter 1 (*SLC16A1*) and the sodium-coupled monocarboxylate transporter 1 (*SLC5A8*) have also been reported.[42] The SCFA–GPCR pathway has been implicated in the regulation of immune response in the gut. The SCFA can modulate the signaling in intestinal epithelial cells by acting through (1) the GPCR pathway that activates the downstream signaling such as mitogen-activated protein kinase, signal transducer and activator of transcription 3 or mammalian target of rapamycin; (2) histone deacetylase (HDAC) inhibition and (3) inflammasome formation (Figure 2).

GPCR pathway

Animal studies have documented that SCFAs activate the expression of GPCR41 and GPCR43 on intestinal epithelial cells, leading to mitogen-activated protein kinase signaling and rapid production of chemokines and cytokines.[43] Studies have investigated a crosstalk between the gut microbiota and the mammalian target of rapamycin signaling pathways mediated by SCFA.[44] SCFA is also involved in mammalian target of rapamycin-mediated AMP production by the intestinal epithelial cells.[45]

HDAC inhibition

In addition to signaling through GPCR, the SCFA can also act as an epigenetic regulator by inhibiting HDAC activity. The HDAC plays a significant role in epigenetic modulation.[46]

Inflammasome formation

The SCFAs also activate the inflammasome pathway that is crucial for maintaining the epithelial barrier integrity. The inflammasome activation is one of the key mechanisms of action of the innate immune system. The pattern recognition receptors, including the NOD (nucleotide-binding oligomerization domain)-like receptors, contribute toward the formation of the inflammasome complex. Conventionally, the NOD, leucin-rich repeat region and pyrin domain-containing protein (NLRP)-3 inflammasome activation require priming through Toll-like receptor (TLR)-4 for its activation. Once primed, the NLRP3 forms the inflammasome complex in association with its adaptor protein, the apoptosis-associated speck-like protein containing a CARD (ASC), and activates caspase-1, which leads to subsequent cleavage and synthesis of functionally active IL-1β and IL-18. The NLRP3 inflammasome pathway along with IL-18 production has been linked to maintenance of gut epithelial integrity and intestinal homeostasis (Figure 2).[47–49] In an experimental colitis model, it has been shown that the binding of SCFAs to GPCR43 and GPCR109A on colonic epithelial cells activates the NLRP3 inflammasome pathway.[50] In addition, the microbiota primes these cells through TLR-4 that subsequently leads to IL-18-mediated epithelial repair.[28] The NLRP3-deficient mice displayed reduced levels of cytokines such as IL-1β, IL-10 and transforming growth factor-β along with reduced levels of colonic β-defensin and AMP secretion that led to loss of gut permeability and a predisposition toward inflammatory gut conditions such as Crohn's disease.[49] The SCFA can influence the intestinal epithelial cell function through GPCR-dependent or GPCR-independent interactions with the cells and preserve the integrity and barrier function. Together, these findings reveal that SCFA can significantly influence the intestinal epithelial cell functions.

MICROBIOTA-DEPENDENT EFFECT ON IMMUNE CELLS

In addition to epithelial cells, the SCFA can also modulate the functionality of immune cells associated with the gut. Studies have documented the beneficial effect of SCFA on the intestinal DCs, macrophages and regulatory T cells (Tregs).

Effect of SCFA on dendritic cells

DCs are antigen-presenting cells and play a crucial role in regulating immune responses to foreign as well as self-antigens. DCs, when encountering an antigen or inflammatory stimuli, undergo maturation evidenced by functional and phenotypic changes. The intestinal DCs are located within the gut-associated lymphoid tissue and also distributed across the LP. They express pattern recognition receptors such as TLR-2 and TLR-4. The prebiotics or their fermentation by-products can bind to these receptors and trigger the maturation process that involves upregulation or downregulation of membrane molecules, namely, CD83, CD86, HLA-DR and DC-SIGN, and also induce the secretion of cytokines. The activation of DCs through particular pattern recognition

receptors defines the polarization of the effector T cells to either T-helper 1 (Th1), Th2, Th17 or Treg phenotype.[51]

The human DCs (both primary and the monocyte derived) express GPCR41 and GPCR109 on the surface. The analysis of human monocyte-derived DC revealed that butyrate and propionate elicit a specific response by inhibiting IL12p70 and IL-23 involved in cytotoxic T-lymphocyte activation, leading to dampening of DC-induced cytotoxic T-lymphocyte activation.[52] Under *in vitro* conditions, butyrate and propionate have also been shown to downregulate the LPS-induced costimulatory molecules CD83, CD80, CD40 and chemokine receptors and skew polarization of the naïve T-cell toward IL-10-producing type 1 Tregs that contribute toward resilience.[53] In addition, propionate and butyrate can enter the DC precursor cell through sodium-coupled monocarboxylate transporter Slc5a8 and inhibit DC maturation through HDAC inhibition.[54] Overall, the findings reveal that SCFAs can modulate DC functionality by inhibiting TLR-4-induced proinflammatory pathways, thereby leading to a regulatory response or blocking the generation of DC from bone marrow stem cells through HDAC inhibition. Mechanistic studies have established that prebiotic oligosaccharides such as the inulin-type fructans target the gut DC through TLRs, NOD-like receptors, C-type lectin receptors and galectins that eventually leads to pro- and anti-inflammatory cytokine release.[55]

Effect of SCFAs on monocytes and macrophages

Macrophages are strategically positioned within the subepithelial region of the gut where antigens or toxins may traverse the intestinal epithelium and jeopardize the barrier integrity. They specialize in phagocytosis and scavenging of potentially harmful microbes, apoptotic cells and cellular debris. The increased activity of peritoneal macrophages, as shown by enhanced superoxide anion production and phagocytosis, has been demonstrated in response to dietary inulin and FOS.[56]

Butyrate has been demonstrated to regulate macrophage polarization through epigenetic modulations. It also downregulates LPS-induced proinflammatory mediators, including nitric oxide, IL-6 and IL-12p40. Interestingly, these effects were found to be independent of the TLR signaling and activation of GPCR and attributed to inhibition of HDAC.[57] The epigenetic mechanisms are known to affect the phenotypic plasticity of macrophage. Butyrate has been shown to facilitate M2 macrophage polarization both *in vitro* and *in vivo* in experimentally induced colitis mice models. *In vitro* experiments have demonstrated that supernatant from butyrate-treated M2 macrophage increased the migration and enhanced the wound closure rate of lung epithelial cells. Mechanistic studies reveal that butyrate enhances IL-4-induced signal transducer and activator of transcription 6 phosphorylation in M2 macrophages by inhibition of *HDAC1* gene expression and increasing H3K9 acetylation in bone marrow-derived macrophages.[58] These findings highlight differential signaling pathways implicated in intestinal tolerance by skewing the polarization of the LP macrophages toward anti-inflammatory phenotype and downregulation of proinflammatory factors.

Effect of SCFAs on T cells

Naïve CD4 T cells can differentiate into many subtypes, including Th1, Th2, Th17 or Tregs. An increasing body of evidence suggests that nutrients and metabolites such as the SCFA may regulate T-cell differentiation.[59–63] Butyrate has been demonstrated to impact $CD8^+$ T-cell memory. Experiments performed in germ-free mice have implicated the role of microbiota in promoting survival of $CD8^+$ T cells as memory cells.[64]

Animal studies have confirmed that consumption of prebiotics does not alter T-cell number but increase the response to mitogen stimulation and enhance cytokine secretion.[65] Another study demonstrated that FOS and inulin consumption do not elicit an increase in the CD4-to-CD8 ratio but skewed the polarization toward a Th1 type of immune response.[66] These studies imply that consumption of prebiotics may not have any effect on the T-cell number but improves the functionality and memory of the existing T cells.

One of the subsets of T cells is Tregs that are generated from naïve T cells in the periphery, generally located at mucosal surfaces that interface with the environment. They can also be transformed by transforming growth factor-β *in vitro* and enabled by forkhead box P3 (FOXP3; induced Tregs). Consumption of prebiotics has been linked with Treg activity *in vivo*. The involvement of Tregs in the anti-inflammatory effect of prebiotic has been demonstrated by a study involving the transfer of tolerance from prebiotic-fed mice to recipient mice through adoptive transfer of splenocytes. Subsequently, partial depletion of Tregs made the recipient mice sensitive to allergic response, implicating the role of Tregs in immune tolerance induced by a prebiotic diet.[67] In addition, studies in non-obese diabetic mice have identified the role of xylooligosaccharide (XOS) in the activation of ($CD69^+$) Tregs in the local lymph nodes.[26] A study conducted in female non-obese diabetic mice fed with long-chain and short-chain inulin-type fructans for 24 weeks showed that the long-chain fructans modulated the T-cell responses, by increasing the number of

$CD25^+FOXP3^+CD4^+$ Tregs and decreasing the number of $IL17A^+CD4^+$ Th17 cells.[68] Administration of short-chain GOS and long-chain FOS to female Balb/c mice resulted in an increased percentage of Tregs.[69] The Tregs express GPCR43 and GPCR109A that engage SCFAs, resulting in induction, expansion and trafficking of the cells.[70] Short-chain fatty acids have also been demonstrated to regulate the size and function of the colonic Treg pool and protect against colitis through a GPCR43-dependent pathway in mice.[71] This study demonstrated that the effect of SCFAs on Treg cell expansion was abrogated in mice lacking GPCR43.[72] Moreover, SCFA induction also involves inhibition of HDACs.[61,70] HDAC inhibition increases the production and function of $FOXP3^+$ Tregs through acetylation of FOXP3. Butyrate treatment increases acetylation of histone H3 lysine 27 (H3K27) at the FOXP3 promoter that leads to increased FOXP3 induction.[73]

Human γδ T cells are known to play a dual role by acting as antigen-presenting cells and directly activating $CD4^+$ T cells in response to microbial as well as some plant-/tea-derived antigens, for example, theanine.[74] The γδ T cells represent a major innate immune cell population in the blood, skin and intestinal epithelium that is involved in the maintenance of gut homeostasis and regulation of inflammation. They are instrumental in triggering early acute inflammatory response in response to any insults to the intestinal barrier integrity and prevent the penetration of microbiota across the impaired intestinal mucosa.[75] Mechanistically, the γδ T cells could sense pathogen-associated molecular patterns through TLRs, thereby indicating a probability of its modulation and expansion by bacterial products.[76,77] Certain food ingredients are also reported to modulate the functionality of these T-cell subsets.[78] The role of prebiotics in regulating the activity of this specialized T-cell subset has not been investigated so far. It is, however, highly probable that the microbial metabolites or antigens may exert direct or indirect effects on γδ T cells by activating the DCs that in turn can regulate γδ T cells via cell-to-cell contact.[79]

Effect of SCFAs on natural killer cells

NK cells are part of the innate immune system and play an important role in antiviral response and tumor immunosurveillance. Hence, it is important to maintain the optimal functionality of NK cells.[80] The NK cells can be directly activated by the invading virus or the tumor or indirectly by DCs. Priming of NK cells eventually leads to inflammatory response and mitigation of the invading pathogen or tumor cells. The NK cells pertaining to the gut are different from those present in the systemic circulation. They interact with gut-associated epithelial cells, fibroblasts, macrophages, DCs and T cells and thereby modulate the immune response. The gut-derived NK cells produce interferon-γ (IFNγ) in response to pathogenic invasion that stimulates recruitment of additional circulating NK cells, leading to amplification of the inflammatory response. The NK cells secrete cytokines such as IFNγ, IL-17 and TNF-α that aids in the maintenance of intestinal barrier integrity.[81] The NK cells use different TLRs (TLR-2, TLR-3, TLR-4 and TLR-9) to interact with whole bacteria or their products to elicit an inflammatory response.[82]

In vivo studies have documented that SCFAs, for example, butyrate and acetate, can enhance NK cell cytotoxicity.[83] Intravenous supplementation with SCFAs has been shown to greatly enhance NK cell activity in rats. Feeding of a prebiotic diet enhances NK cell activity *in vivo*. Soybean oligosaccharides induced a significant increase in the activity of NK cells as compared with that observed in the control group.[84] β-1,4-Mannobiose has been shown to exert increased NK cell activity relative to LPS control in mice.[85] GOS has been reported to significantly reduce colitis severity by increasing the percentage of NK cells in the spleen and mesenteric lymph nodes following infection. It also stimulated the trafficking of NK cells in the blood by stimulating the chemokine receptor CCR9.[86] Isomaltooligosaccharide-fed mice exhibited an increased proportion of NKT cells in the liver mononuclear cells in the spleen.[87] Studies using symbiotic combination such as FOS-enriched inulin alone or in combination with probiotics such as *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 enhanced NK cell activity in the blood.[88] This evidence shows that prebiotics and bacterial metabolites enhance NK cell activity; this information is crucial particularly for the aged population where the decline in NK cell function renders them prone to infection.[89]

Effect of SCFAs on neutrophils

Neutrophils are innate immune cells that protect the host by targeting antigens that cross the epithelial barrier. They release cytokines and chemokines that further activates the adaptive immune response. Inflammatory conditions trigger the expression of GPCRs on these cells that renders them sensitive to anti-inflammatory activities induced by SCFAs. GPCR43 is highly expressed on neutrophils which indicates the potential of dietary fiber intake on neutrophil recruitment during inflammatory responses. SCFAs are known to affect the intracellular pH, calcium concentration and other effector functions of neutrophils such as production of reactive oxygen species, phagocytosis and chemotaxis.[90,91] Butyrate has been

shown to inhibit neutrophil reactive oxygen species release and phagocytosis.[92,93] Propionate and butyrate diminished TNF-α, Cytokine- induced neutrophil chemoattractant- 2 alpha beta (CINC-2αβ) and nitric oxide production by LPS-stimulated neutrophils through inhibition of HDAC activity and NF-κB activation.[94] The role of SCFA and GPCR43 interaction in the inhibition of neutrophil chemotaxis has been extensively studied. Studies using GPCR43-deficient mice have reported that the SCFA also functions to mitigate inflammation by inhibiting the migratory potential of neutrophil via GPCR43.[95] The intravital imaging of neutrophil migration and adhesion potential in the small intestine of GPCR43-deficient mice revealed a time-dependent decrease in the intravascular neutrophil rolling and adhesion with a concomitant increase in recruitment to LP in response to LPS. GPCR43-deficient leukocytes also demonstrated increased migration into the peritoneal cavity following *N*-formyl methionyl-leucine-phenylalanine challenge. This LPS- and *N*-formyl methionyl-leucine-phenylalanine-induced neutrophil migration and recruitment were significantly suppressed in wild-type mice that were treated with acetate. In addition, this elevated migration could also be reproduced in germ-free mice through a simple transfer of no-fiber microbiota. These findings implicate GPCR43 and a microbiota composition that allows for SCFA production in modulating neutrophil migration and recruitment during inflammatory responses. Studies have also indicated rapid and transient activation of Rac1/2 GTPases and phosphorylation of ribosomal protein S6 in neutrophils upon GPR43 activation in neutrophils. Further studies using pharmacological inhibitors have implicated phosphatidylinositol-3-kinase-γ, Rac2, p38 and extracellular signal-regulated kinase pathway in GPCR43-dependent chemotaxis of neutrophils.[96] These studies indicate that the crosstalk between neutrophils and the microbiota is mediated by SCFAs, which regulate neutrophil function and restore the magnitude of induced inflammation and vis-à-vis prevents inflammatory responses against the host commensal.

MICROBIOTA-INDEPENDENT EFFECT ON GUT EPITHELIAL CELLS

The prebiotics can affect immune functions in the absence of microbiota and metabolites. This immunomodulatory effect of oligosaccharides is a consequence of its direct effect on cellular signaling pathways. TLR-4 ligation in the intestinal epithelial cells has been demonstrated to induce growth-related oncogene (GRO-α)-α, monocyte chemoattractant protein 1 and macrophage inflammatory protein 2 secretion through NF-κB activation (Figure 2).[97] TLR-2 located on intestinal epithelial cells has also been shown to be a target of β2 → 1-fructan.[98] *In vitro* assays performed on a porcine jejunum epithelial cell line (IPEC-J2) revealed that *Platycodon grandiflorus* fructan, an inulin-type fructan, induced a significant increase in the messenger RNA levels of anti-inflammatory cytokines such as IL-4 and IL-10 along with a minor increase in the proinflammatory factors such as IL-1β and TNF-α.[99] The immunomodulatory capacities of oligosaccharide mixtures (scGOS/lcFOS and scFOS/lcFOS) were investigated on HT29 cells cocultured with peripheral blood mononuclear cells derived from peanut-allergic patients. It was found that the oligosaccharide mixtures were effective in significantly enhancing IFNγ and IL-10, while decreasing IL-13 and TNF-α production, suggesting modulation of both proinflammatory and regulatory response.[100] Caco-2 cells when exposed to scFOS exhibited increased expression of the anti-inflammatory cytokines such as IL-10 and transforming growth factor-β.[101] The HMOs have also been implicated in reducing the signatures of inflammation in intestinal epithelial cells.[102,103] These studies have highlighted the anti-inflammatory effect of prebiotics on the gut epithelial cells that are independent of the microbiota.

MICROBIOTA-INDEPENDENT EFFECT ON IMMUNE CELLS

Apart from their effect on intestinal epithelial cells, the prebiotics induced direct effect on the signaling pathways of immune cells as well. In a coculture system, it has been observed that intestinal epithelial cells primed with resistant starch modulated the functionality of DCs and skewed them toward a more regulatory phenotype.[104] In line with the epithelial cells, the predominant effect of prebiotics on the monocyte activation is also primarily mediated through TLR-4 ligation that leads to induction of TNF-α, growth-related oncogene-α (GRO-α) and IL-10 (Figure 2). Studies using pharmacological inhibitors have implicated the NF-κB, mitogen-activated protein kinase and phosphatidylinositol-3-kinase pathways in monocyte activation.[105] scGOS/long-chain FOS has been shown to act via TLR-4 and induce IL-10 in monocyte-derived DCs and a possible induction of Tregs.[106] *Platycodon grandiflorum*, an inulin-type polyfructose, has been reported to induce an immunosuppressive environment in *ex vivo* peripheral blood mononuclear cell by promoting FOXP3 gene expression and IL-10 secretion.[107]

The effect of oligosaccharides is mediated primarily through the TLR signaling such as the NF-κB, mitogen-activated protein kinase and extracellular signal-regulated

kinase pathways,[107] but studies have also documented their interaction with other recognition molecules such as peroxisome proliferator-activated receptor-γ that renders an anti-inflammatory response. Oligosaccharides such as α3-sialyllactose and FOS have been reported to induce activation of peptidoglycan recognition protein 3 (PGlyRP3) through increased expression of peroxisome proliferator-activated receptor-γ, a member of nuclear receptor superfamily, that inhibits NF-κB signaling and the expression of proinflammatory cytokines such as IL-12, IL-8 and TNF-α, creating an anti-inflammatory milieu.[108] In addition, peroxisome proliferator-activated receptor-γ has been reported to interfere with and inhibit TLR-4-mediated proinflammatory signaling pathways including extracellular signal-regulated kinase 1/2, PKC and NF-κB (Figure 3).[109] These reported interactions of prebiotics with the immune system add to the complexities of the signaling events, leading to either a pro- or an anti-inflammatory response. The anti-inflammatory effect of prebiotics becomes more evident under inflammatory disorders such as Crohn's disease or certain allergies, wherein the prebiotics have been shown to modulate the DCs and monocytes to secrete IL-10, which leads to a Treg response.[110] In healthy individuals, inflammation resulting from a high-fat diet can be attenuated by consumption of XOS in combination with inulin.[111] A recent *in vitro* finding revealed that the HMOs attenuate LPS-induced immune activation of human monocyte-derived DCs by modulation of TLR-4 and DC sign receptors that maintain a tolerogenic milieu.[112] All these findings suggest the potential anti-inflammatory effect of prebiotic oligosaccharides. However, further research is warranted to resolve the signaling event elicited by the direct interaction of prebiotic with an immune cell. Various *in vitro* and *in vivo* studies have documented the direct modulation of DC and macrophage functionality.

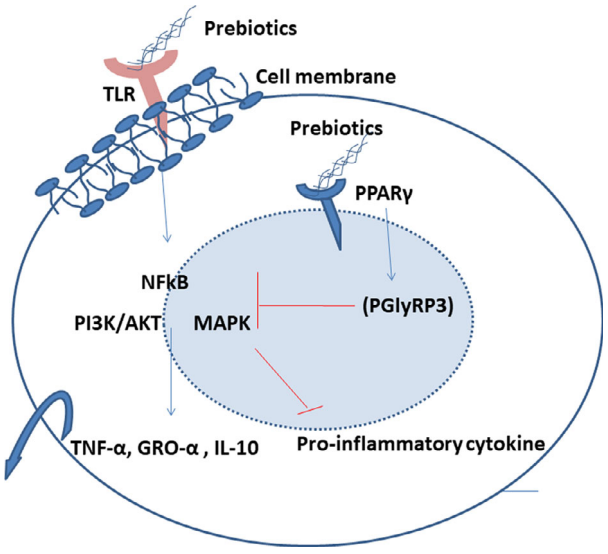

Figure 3. Schematic representation of the direct action of prebiotics on immune cell. Prebiotic oligosaccharides act as ligands to either TLR-2, TLR-4 or TLR-5 and induce NF-κB, MAPK or ERK signaling pathways that leads to the secretion of pro- and anti-inflammatory cytokines. The prebiotics may also activate the PPARγ and Peptido glycan recognition protein 3 (PGlyP3) molecules that inhibit the TLR-mediated signaling pathways that eventually leads to the inhibition of proinflammatory cytokine release. AKT, protein kinase B; ERK, extracellular signal-regulated kinase; GRO-α, growth-related oncogene-α; IL, interleukin; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; PPARγ, peroxisome proliferator-activated receptor-γ; TLR, Toll-like receptor; TNF, tumor necrosis factor.

Dendritic cells

In vitro studies provide evidence for the independent interaction of nondigestible oligosaccharides with TLR-4 expressed on human monocyte-derived DCs that results in the secretion of IL-10 and induction of FOXP3-positive Tregs in an allogenic T-cell stimulation assay.[106] *P. grandiflorus* fructan has been demonstrated to induce maturation of DCs, as indicated by an increase in inflammation-associated cytokine markers linked to the maturation process, for example, IL-1, IL-6, IL-10, IL-12, IFNγ. Data using DCs isolated from TLR-4 wild-type mice and endotoxin-resistant TLR-4 mutant mice strongly implicate the TLR-4 receptor in *P. grandiflorus* fructan-induced DC maturation.[107] The TLR-4 and DC sign receptors are also modulated by the HMOs that promote a tolerogenic environment with elevated levels of IL-10, IL-27 and IL-6 and increased number of Treg from naïve T cell that is crucial for the development of neonatal immunity.[112] Contrasting results were reported in another study investigating the effect of HMOs such as 6′-sialyllactose and 2′-fucosyllactose along with GOS on monocyte-derived DCs. This study highlighted that the tested combination of HMOs did not induce secretion of IL-10, IL-6 or IL-8 and had no effect on the differentiation of monocytes into DCs. The induced levels of IL-6 and IL-10 that were observed in the case of 6′-sialyllactose exposure were attributed to the presence of LPS as contaminant.[113] The authors in this study have used commercially available oligosaccharides as representative of HMO, whereas the first study reported results of oligosaccharides isolated from pooled human milk that mimics an *in vivo* condition more closely. The discrepancy in the results obtained in the two studies can be attributed to the difference in composition of the HMOs. The oligosaccharides isolated from pooled human milk comprise a pool of close to 140 different oligosaccharides

and may demonstrate differential response when compared with the effect of oligosaccharides used in isolation. The direct effect of prebiotics on the DCs has also been confirmed by *in vivo* studies conducted in germ-free mice, wherein treatment with scβ2 → 1-fructans demonstrated an increase in $CD11b^{-}CD103^{-}$ DCs in the mesenteric lymph nodes and a decrease in $CD11b^{+}CD103^{+}$ DCs, thereby implying that consumption of a prebiotic diet promotes migration of $CD11b^{-}CD103^{-}$ DCs from the LP to the mesenteric lymph nodes to induce Tregs in the absence of microbiota.[114]

Macrophages

Several *in vitro* studies have documented the immunomodulatory activity of prebiotics in monocyte activation. Konjac oligosaccharides induced activation of murine macrophage RAW 264.7 cell line to secrete nitric oxide via the inducible nitric oxide synthase pathway and cytokines IL-10 and IL-6.[115] Agave fructans induced activation of monocyte function by inducing the synthesis of nitric oxide in combination with probiotic strains, for example, *Lactobacillus casei* and *B. lactis*.[116] It is shown that FOS and inulin induced secretion of TNF-α, IL-6 and IL-10 from mouse splenocytes but inhibited LPS-induced secretion of IFNγ and IL-17 through TLR-mediated signaling pathways.[105] FOS also significantly enhanced nitric oxide production by peritoneal exudate cells isolated from Wistar rats, and intracellular free radicals production and phagocytic activity of peritoneal exudate cells isolated from mice.[117]

Prebiotics, such as fucoidan, have been assessed for their effect on the functionality of monocytes and macrophages and have been reported to induce adhesion, migration and secretion of matrix metalloproteinases by these cells.[118] Data from *in vivo* studies with different types of dietary fibers reveal that these prebiotic fibers exert distinct immunological effects. The effect of XOS on mucosal inflammation in non-obese diabetic mice was studied. It was observed that in mice fed with XOS there was an increase in the abundance of anti-inflammatory M2 macrophages but not in that of the classical proinflammatory M1 type of macrophage.[26] This finding is relevant as it documents the ability of prebiotics to skew the polarity of macrophage to the M2 anti-inflammatory phenotype.

PRO- AND ANTI-INFLAMMATORY PATHWAYS AFFECTED BY PREBIOTICS AND THEIR THERAPEUTIC POTENTIAL

The change in dietary pattern brought about by increased consumption of fat and low intake of fibers has resulted in increased susceptibility to gut-associated inflammatory disorders. In this context, consumption of prebiotics can modulate and restore healthy microbiota. The SCFA strongly impacts the gut epithelial and immune cells and aids to combat inflammation and has been explored as a promising avenue for therapeutic intervention in the treatment of inflammatory disorders. Disorders such as Crohn's disease and ulcerative colitis are believed to involve dysregulated proinflammatory response, with one of the causative factors being dysbiosis of gut.[119]

Crohn's disease involves high expression of proinflammatory cytokines such as IL-12/23, TNF-α, IFNγ and IL-17.[120] As discussed in the previous section, prebiotics can activate the NF-κB pathway by priming through TLR-4, resulting in secretion of proinflammatory cytokines along with regulatory IL-10.[105] They can alternatively activate the peroxisome proliferator-activated receptor-γ pathway that mitigates the proinflammatory response elicited by NF-κB signaling.[108] The SCFA, by contrast, activates the NOD-like receptors and the inflammasome pathway that induces secretion of IL-10 and transforming growth factor-β along with AMPs.[42,121] The mutation in the NOD pathway has been implicated in the etiology of Crohn's disease, where loss-of-function mutations result in a loss of tolerance to commensals and dysbiosis.[122] Prebiotics, by virtue of their ability to impact the growth of probiotic bacteria, help in rebiosis; in addition, the ability to balance the pro- and anti-inflammatory response further bestows them with medicinal benefits. In light of this evidence, a number of human intervention studies have been performed to validate the effect of prebiotics in diseased and healthy individuals.

HUMAN CLINICAL STUDIES

Several human clinical trials have been performed using dietary interventions with prebiotics to understand their role in regulating the immune response (Table 2). Few intervention studies have been conducted in healthy individuals to decipher the importance of prebiotics under basal conditions. One such study revealed that supplementation of β2-1 fructan (3 × 5 g per day) in the diet for two 28-day treatments separated by a 14-day wash-out period decreased circulating percentages of $CD282^{+}/TLR2^{+}$ myeloid DCs and *ex vivo* responsiveness to a TLR-2 agonist along with a decline in the serum levels of IL-10 and increase in proinflammatory cytokines,[123] indicating a proinflammatory response. It was speculated that this may be a result of loss of barrier integrity and translocation of gut microbiota during the supplementation phase as indicated by an increase in serum LPS. No major health benefit was noted in the

Table 2. Overview of clinical studies on the effect of prebiotics on innate immune cells

Intervention	Condition	Study duration	Immunological parameters	Trial outcome	Ref
β2-1 fructan (3 × 5 g per day)	Healthy	Two 28-day supplementation with 14-day wash-out in between	• Increase in the % of circulating $CD282^+/TLR2^+$ myeloid DC • *Ex vivo* responsiveness to a TLR-2 agonist • Decrease in serum levels of IL-10 • Increase in proinflammatory IL-4 and GM-CSF	• No major health benefit • Increased proinflammatory response, possibly a result of loss of barrier integrity • Minor gastrointestinal discomfort and headaches noted	123
β2-1 fructan (8 g per day)	Healthy adults	4 weeks	• No effect on T-cell activation, proliferation and production of cytokines • No effect on neutrophil and monocyte phagocytosis and oxidative burst • No effect on NK cell activity	• No effect on immunity • Increased bowel movement and flatulence	124
B-GOS (5.5 g per day)	Healthy elderly individuals	10 weeks	• Improved NK cell activity	Significantly improved immune functions	125
Inulin (10 g per day)	T2 Diabetic females	8 weeks	• Reduced levels on inflammatory markers such as high-sensitive c-reactive protein, TNF- α and LPS	Controlled inflammation and metabolic endotoxemia	126
FOS (15 g per day)	Crohn's disease	3 weeks	• Increase in expression of $IL\text{-}10^+$ DC • Increase in TLR-2 and TLR-4 levels • Reduced proportion of $IL\text{-}6^+$ DC	Improvement in disease activity	110
FOS (15 g per day)	Crohn's disease	4 weeks	• Reduced proportion of $IL\text{-}6^+$ DC • Increase in expression of $IL\text{-}10^+$ DC	No effect on disease condition	13
FOS-enriched inulin	T2DM	12 weeks	• Increased serum levels of IL-4 and decreased IL-12 and IFNγ • No significant changes in $CD3^+$, $CD4^+$, $CD8^+$ and $CD11b^+$ T-cell counts	• Improvement in glycemic status, lipid profile and immune markers • Reduced body mass index blood pressure	132
GOS (8 g per day) + *B. lactis* Bi-07	Healthy elderly adults	Four 3-week periods separated by 4-week wash-out periods	• No change in the number of monocytes • Increase in phagocytic efficiency • Increase in *ex vivo* proliferation and IFNγ production of PBMC		127
XOS 8 g + 10^9 CFU Bi-07 per day	Healthy adults (25–65 years)	3 weeks	• Bi-07 lowered IL-4 and increased IL-6 secretion • XOS lowered CD16/56 on NK T cells and IL-10 secretion • XOS and Bi-07 reduced the expression of CD19 on B cells	• XOS induces bifidogenesis, improves plasma lipid profile • XOS and Bi-07 have immunostimulatory effects,	128
GOS (3.8 g per day) + *Lactobacillus rhamnosus*,	Healthy young adults	2 weeks	• Increased PBMC proliferation and IFNγ secretion	Improved immunity	129

(*Continued*)

Table 2. Continued.

Intervention	Condition	Study duration	Immunological parameters	Trial outcome	Ref
Propionibacterium freudenreichii and *Bifidobacterium breve*					
Soluble corn fiber + *L. rhamnosus* and pilus-deficient *L. rhamnosus*	Healthy elderly women	3 weeks	• Increase in NK cell activity • Decrease in C-reactive protein	Reduced total cholesterol and LDL-cholesterol	130
Inulin + *L. rhamnosus* and *Bifidobacterium lactis* Bb12	Polypectomized individuals and colon cancer patients	12 weeks	• No effect on phagocytic and respiratory burst activity of neutrophils and monocytes; lytic activity of natural killer cells • Increased capacity of PBMC to produce IFNγ	Minor stimulatory effects on the systemic immune system	131

CFU, colony-forming unit; DC, dendritic cell; FOS, fructooligosaccharide; GM-CSF, granulocyte-macrophage colony-stimulating factor; GOS, galactooligosaccharides; IFN, interferon; IL, interleukin; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NK, natural killer; PBMC, peripheral blood mononuclear cell; ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus; TLR, Toll-like receptor; TNF, tumor necrosis factor; XOS, xylooligosaccharide.

trial. Minor side effects related to gastrointestinal discomfort and incidences of headaches were reported which may be attributed to the high daily dose (15 g per day). Another trial aimed at investigating the effect of supplementation of β2-1 fructan (8 g per day) on immunological parameters studied the effect of feeding the prebiotic to healthy volunteers for 4 weeks. The results showed no alterations in the number of markers of systemic innate, humoral and T-cell-mediated immunity. Lomax *et al.*[124] speculated that the prebiotic did not alter the systemic immune parameters because the participants were healthy individuals with optimum immune response. In the case of elderly individuals, it has been observed that supplementation of prebiotics induces an immunomodulatory effect. Vulevic *et al.*[125] investigated the effect of GOS on markers of immune function in elderly (age 65–80 years) volunteers. The intervention period comprised 10 weeks daily dosing with 5.5 g of GOS and a 4-week wash-out period in between. Administration of prebiotics increased levels of IL-10, IL-8 and NK cell activity and reduced IL-1β secretion. These results suggest that feeding GOS reduces chronic inflammation associated with age by increasing the levels of IL-10 and lowering of IL-1β. Increased NK cell activity and IL-8 secretion, a neutrophil chemotactic factor, signifies strengthening of innate immunity in defense against infections. In addition, the prebiotics play a role in modulating the inflammation that is associated with pathological conditions. In women with type 2 diabetes consumption of inulin (10 g per day) for 8 weeks reduced levels of inflammatory markers such as high-sensitive C-reactive protein, TNF-α and LPS.[126] In patients with active Crohn's disease 3 weeks of supplementation with a mixture of oligofructose and inulin (15 g per day) resulted in an improvement in disease condition along with modifications of the innate immune system to moderate inflammation, such as increased IL-10 and TLR expression in mucosal DCs.[110] Benjamin *et al.*[13] reported that consumption of FOS (15 g per day) for 4 weeks by patients with Crohn's disease modulated DC functionality by skewing them toward the anti-inflammatory phenotype by reducing IL-6 and increasing IL-10 expression. Unlike the previous study, here consumption of prebiotics showed no clinical benefit in patients. Overall, the data reveal that consumption of prebiotics shows no significant effect on the immune system of the healthy individuals; however, elderly or persons with underlying pathophysiological conditions may benefit from a prebiotic diet.

Dietary intervention studies have been designed to include combinations of prebiotics along with probiotic bacteria or synbiotics. A randomized, double-blind, placebo-controlled, cross-over human clinical trial

involving elderly healthy adults was conducted, wherein the recruits consumed prebiotic GOS (8 g per day), probiotic *B. lactis* Bi-07 and their combination (Bi-07 + GOS) for four 3-week periods separated by 4-week wash-out periods. The effect on immunity was determined by studying the phagocytic potential and oxidative burst in monocytes and granulocytes. The results revealed that the number of monocytes engaged in phagocytosis was not significantly altered, but the efficiency of phagocytosis was greatly enhanced in the probiotic and synbiotic group. Prebiotic alone also induced a significant increase in the phagocytic activity of the monocytes but not to the extent observed in the other two groups.[127] This study shows that prebiotics can positively influence the functionality of monocytes and macrophages, and that they act in synergy with probiotic bacteria. Consumption of XOS (8 g per day) and *Bifidobacterium animalis* subsp. *lactis* (Bi-07) for 3 weeks by healthy volunteers demonstrated proinflammatory effects. There were no significant effects on the measures of phagocytosis or oxidative burst but there was an increased expression of IL-6 and lowered expression of IL-10 and IL-4.[128] In lines with these findings, GOS (3.8 g per day) in combination with probiotics, when administered to healthy men for 2 weeks, resulted in increased proliferation and IFNγ production in resting as well as activated peripheral blood mononuclear cells *ex vivo*.[129] In elderly populations the administration of prebiotic soluble corn starch in symbiotic combination with *L. rhamnosus* GG increased NK cell activity and reduced IL-6.[130] This finding further reiterates that prebiotic improves chronic age-related inflammation and innate immune response in the elderly population. In polypectomized individuals and colon cancer patients the effect of consumption of inulin in combination with *L. rhamnosus* GG and *B. lactis* Bb12 on the immune system was studied. The intake of the symbiotic combination did not affect phagocytosis, oxidative bursts and the NK cell activity in the study groups compared with the placebo control group. However, modulation in the levels of IL-2 and IFNγ were noted in *ex vivo* mitogen (ConA, LPS and phytohemagglutinin) stimulated peripheral blood mononuclear cell cultures.[131] These studies show that the consumption of synbiotics did not significantly alter the systemic immunological parameters; however, in all probability one cannot disregard the impact on the gut-associated immunity that has not been investigated in these studies. The clinical trials conducted in a healthy young population reveal that prebiotics are more effective in modulating the immune response when used in combination with probiotics. The symbiotic combination helps in improving the immunological parameters alongside improvement in serum lipid profile, glucose levels, body mass index and blood pressure. In the elderly population or in the presence of any underlying pathologic condition associated with chronic inflammation such as IBD, ulcerative colitis, diabetes, obesity, prebiotics either alone or in association with probiotics induce an anti-inflammatory effect. Hence, studies involving prebiotics both alone and in symbiotic combinations are emerging and promising to pave the way for dietary therapies in combating chronic debilitating diseases.

Challenges of prebiotic therapy

Although prebiotics have been explored in an increasing number of clinical trials, the conclusions drawn are not always clinically relevant. In patients with Crohn's disease, two trials investigated the effect of feeding prebiotic FOS; in one study supplementation with 15 g per day for 3 weeks showed improvement in immunological markers,[123] whereas in the other study a 4-week supplementation showed no effect on the immunity parameters.[124] In both the studies, no clinical efficacy was noted and minor side effects related to the severity of flatulence and rumbling of the gut, and headaches were observed among volunteers. Two clinical studies on IBD patients have reported no benefits upon supplementation with FOS 6 g per day for 4 weeks and 20 g per day for 6 weeks.[133,134] By contrast, an improvement in symptoms of IBD has been reported with FOS (5 g per day for 6 weeks) in a randomized controlled trial involving 105 patients.[135] Therefore, clinically, the evidence of prebiotic effectiveness is still controversial; a meta-analysis of randomized controlled trials revealed that consumption of prebiotics did not improve gastrointestinal symptoms; however, the variety and dose modulated the severity of symptoms.[136] A recent meta-analysis that evaluated the effects of prebiotics and synbiotics on irritable bowel syndrome symptoms stated lack of evidence for significant impact on irritable bowel syndrome symptoms.[137] As a matter of fact, there is some evidence that higher doses may have a negative effect on symptoms.[123,124,134,135] Therefore, fixing of therapeutic effective dose is also challenging as higher doses, albeit efficacious, are associated with side effects. On the clinical front there still exists a paucity of information on the benefits of prebiotic intervention for treating disease conditions.

CONCLUSION

The gut microbiome is strongly implicated for modulating the pro- and anti-inflammatory immune response that is crucial for preserving the delicate balance

between health and disease. The human gut microbiome is largely unexplored and the Human Gut Microbiome Initiative has been undertaken as an extension of the human genome project to decipher the microbial communities and predict predisposition toward various ailments such as diabetes, gastrointestinal disorders, atopic disorders and other immunopathological conditions. Aligned to this, the prebiotics have been extensively explored for regulating the gut microbiome and their influence on the health and wellness of the host. The 16S ribosomal RNA-based molecular biology technologies are being adopted to study the changes in intestinal microbiota as a result of consumption of different prebiotics. These studies have clearly implicated the importance of prebiotic fibers directed at influencing the gut microbiota for a beneficial effect on human health.[138] In addition, the prebiotics harbor the potential to either directly or indirectly modulate the host immune system. This immunomodulatory property of the prebiotics has been exploited to develop potential applications in health and wellness products as well as adjunct immunomodulatory therapy for a wide variety of chronic disease conditions. Future studies are therefore warranted to provide a more comprehensive understanding of the mechanism of action of prebiotics on the components of the innate and acquired immune system.

ACKNOWLEDGMENTS

The project is supported by Tata Chemicals Ltd. The authors acknowledge the contribution of Ms Risha Banerjee in reviewing the final document.

CONFLICT OF INTEREST

Tata Chemicals Ltd is the manufacturer of prebiotics (Fossence/Gossence). None of the references cited in this article are linked to experiments associated with these products.

AUTHOR CONTRIBUTIONS

Radha Pujari: Conceptualization; Data curation; Writing-original draft; Writing-review & editing. **Gautam Banerjee:** Conceptualization; Resources; Supervision; Writing-review & editing.

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